

TAU-CONOTOXIN PEPTIDES

CROSS-REFERENCE TO RELATED APPLICATION

[0001] The present application is a divisional application of U.S. Application No. 09/497,491, filed February 4, 2000, which claims benefit of U.S. Provisional Patent Application No. 60/118,642, filed February 4, 1999. The disclosures of the above identified applications are hereby incorporated by reference.

[0002] This invention was made with Government support under Grant No. PO1 GM48677 awarded by the National Institute of General Medical Sciences, National Institutes of Health, Bethesda, Maryland. The United States Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] The invention relates to relatively short peptides (termed τ -conotoxins herein), about 10-20 residues in length, which are naturally available in minute amounts in the venom of the cone snails or analogous to the naturally available peptides, and which preferably include two disulfide bonds.

[0004] The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated by reference, and for convenience are referenced in the following text by author and date and are listed alphabetically by author in the appended bibliography.

[0005] The predatory cone snails (*Conus*) have developed a unique biological strategy. Their venom contains relatively

small peptides that are targeted to various neuromuscular receptors and may be equivalent in their pharmacological diversity to the alkaloids of plants or secondary metabolites of microorganisms. Many of these peptides are among the smallest nucleic acid-encoded translation products having defined conformations, and as such, they are somewhat unusual. Peptides in this size range normally equilibrate among many conformations. Proteins having a fixed conformation are generally much larger.

[0006] The cone snails that produce these peptides are a large genus of venomous gastropods comprising approximately 500 species. All cone snail species are predators that inject venom to capture prey, and the spectrum of animals that the genus as a whole can envenomate is broad. A wide variety of hunting strategies are used, however, every *Conus* species uses fundamentally the same basic pattern of envenomation.

[0007] Several peptides isolated from *Conus* venoms have been characterized. These include the α -, μ - and ω -conotoxins which target nicotinic acetylcholine receptors, muscle sodium channels, and neuronal calcium channels, respectively (Olivera et al., 1985). Conopressins, which are vasopressin analogs, have also been identified (Cruz et al., 1987). In addition, peptides named conantokins have been isolated from *Conus geographus* and *Conus tulipa* (Mena et al., 1990; Haack et al., 1990).

[0008] Chronic or intractable pain, which may result from degenerative conditions or debilitating diseases, is currently treated with a variety of analgesic compounds, often opioid compounds such as morphine. Likewise, neuropathic pain, typically a chronic condition attributable

to injury or partial transection of a peripheral nerve, is also conventionally treated with opioid compounds such as morphine.

[0009] Conventional therapies for pain produce analgesia--a loss of sensitivity to pain without the loss of consciousness. Opioid compounds have been used widely to produce analgesia, including plant-derived opioids such as morphine, and endogenous opioids such as met- and leu-enkephalins, as well as beta-endorphin.

[00010] Opioid compounds, while effective in producing analgesia for many types of pain, may induce tolerance in some patients. When a patient becomes tolerant, increasing doses of the opioid are required to produce the desired analgesic effect. In addition, these compounds frequently result in a physical dependence in patients, and may have side effects at high doses.

[00011] The analgesic effects and adverse actions of various NMDA receptor antagonists has been shown to vary depending on the site of action and potency of the drug. For example, NMDA receptor antagonists acting at the ion channel in a noncompetitive manner (e.g., MK-801 and phenylcyclidine (PCP)) or competitive inhibitors, show analgesic activity but show motor impairment at equivalent doses. Glycine B-site NMDA antagonists appear to have analgesic activity at doses that do not impair motor function. Conantokins, which are polyamine-site NMDA antagonist compounds have analgesic effects at doses which do not produce overt side effects (PCT published application WO 98/03189).

[00012] It is desired to provide additional compounds which have analgesic properties.

SUMMARY OF THE INVENTION

[00013] The invention relates to relatively short peptides (termed τ -conotoxins herein), about 10-25 residues in length, which are naturally available in minute amounts in the venom of the cone snails or analogous to the naturally available peptides, and which preferably include two disulfide bonds.

[00014] More specifically, the present invention is directed to τ -conotoxin peptides having the general formula I:

Xaa₁-Xaa₂-Xaa₃-Xaa₄-Cys-Cys-Xaa₅-Xaa₆-Xaa₇-Xaa₈-Xaa₉-Cys-Cys-Xaa₁₀-Xaa₁₁-Xaa₁₂-Xaa₁₃-Xaa₁₄-Xaa₁₅-Xaa₁₆-Xaa₁₇-Xaa₁₈-Xaa₁₉ (SEQ ID NO:1), wherein Xaa₁ is des-Xaa₁, Asp, Glu or γ -carboxy-Glu (Gla); Xaa₂ is des-Xaa₂, Gln, Asn, Glu, Trp (D or L), neo-Trp, halo-Trp or any unnatural aromatic amino acid; Xaa₃ is des-Xaa₃, Gly, Ala, Asn or Gln; Xaa₄ is des-Xaa₄, Val, Leu (D or L), Ile, Ala, Gly, Glu, Gla, Asp, Ser, Thr, Phe, Trp (D or L), neo-Trp, halo-Trp (D or L) or any unnatural aromatic amino acid; Xaa₅ is Pro, hydroxy-Pro, Gln, Asn, Glu, Gla, Ala, Gly, Lys, Arg, Ile, Val, homoarginine, ornithine, N-methyl-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys or any unnatural basic amino acid; Xaa₆ is Val, Phe, Thr, Ser, Glu, Gla, Asp, Asn, Gln, Ala, Gly, Ile, Leu (D or L) Met, Pro, hydroxy-Pro, Arg, homoarginine, ornithine, Lys, N-methyl-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, any unnatural basic amino acid or any unnatural aromatic amino acid; Xaa₇ is any Val, Ile, Asn, Leu (D or L), Gln, Gly, Ala, Phe, Glu, Gla, Arg, ornithine, homoarginine, Lys, N-methyl-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, any unnatural basic amino acid or any unnatural aromatic amino acid; Xaa₈ is Ile, Leu (D or L), Met, Thr, Ser, Pro, hydroxy-Pro, Gln, Asp, Glu, Gla, Asn,

Arg, homoarginine, ornithine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, Tyr, nor-Tyr, mono-halo-Tyr, di-halo-Tyr, O-sulpho-Tyr, O-phospho-Tyr, nitro-Tyr, any unnatural basic amino acid, any unnatural aromatic amino acid or any unnatural hydroxy containing amino acid; Xaa₉ is des-Xaa₉, Ala, Gly, Asp, Glu, Gla, Trp (D or L) neo-Trp, halo-Trp (D or L), Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, Arg, homoarginine, ornithine, Tyr, nor-Tyr, mono-halo-Tyr, di-halo-Tyr, O-sulpho-Tyr, O-phospho-Tyr, nitro-Tyr or any unnatural basic amino acid; Xaa₁₀ is des-Xaa₁₀, Ile, Leu (D or L), Val, Glu, Gla, Asp, Thr, Ser, Pro, hydroxy-Pro, Trp (D or L), neo-Trp, halo-Trp (D or L), Phe, any unnatural aromatic amino acid or any unnatural hydroxy containing amino acid; Xaa₁₁ is des-Xaa₁₁, Gln, Asn, Leu (D or L), Ile, Val, Ala, Gly, Trp (D or L), neo-Trp, halo-Trp (D or L), Arg, homoarginine, ornithine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, any unnatural basic amino acid or any unnatural aromatic amino acid; Xaa₁₂ is des-Xaa₁₂, Ala, Gly, Phe, Trp (D or L), neo-Trp, halo-Trp (D or L) or any unnatural aromatic amino acid; Xaa₁₃ is des-Xaa₁₃, Glu, Gla, Asp, Phe or any unnatural aromatic amino acid; Xaa₁₄ is des-Xaa₁₄, Ile, Val or Leu (D or L); Xaa₁₅ is des-Xaa₁₅, Thr, Ser, Arg, homoarginine, ornithine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys or any unnatural basic amino acid; Xaa₁₆ is des-Xaa₁₆, Glu, Gla or Asp; Xaa₁₇ is des-Xaa₁₇, Asn or Gln; Xaa₁₈ is des-Xaa₁₈, Asp, Glu or Gla; Xaa₁₉ is des-Xaa₁₉, Phe or any unnatural aromatic amino acid. The C-terminus may contain a free carboxyl group or an amide group. The halo is preferably bromine, chlorine or iodine, more preferably iodine for Tyr and bromine for Trp. The Cys residues may be in D or L configuration and may optionally be substituted

with homocysteine (D or L). The Tyr residues may be substituted with the 3-hydroxyl or 2-hydroxyl isomers and corresponding O-sulpho- and O-phospho-derivatives. The acidic amino acid residues may be substituted with any synthetic acidic amino acid, e.g., tetrazolyl derivatives of Gly and Ala.

[00015] The present invention is also directed to novel specific τ -conotoxin peptides of general formula I having the formulas:

Phe-Cys-Cys-Xaa₁-Val-Ile-Arg-Xaa₂-Cys-Cys-Xaa₃ (SEQ ID NO:2);

Phe-Cys-Cys-Xaa₁-Phe-Ile-Arg-Xaa₂-Cys-Cys-Xaa₃ (SEQ ID NO:3);

Cys-Cys-Gln-Thr-Phe-Xaa₂-Xaa₃-Cys-Cys-Gln (SEQ ID NO:4);
Xaa₄-Gly-Xaa₃-Cys-Cys-Xaa₅-Xaa₆-Asn-Ile-Ala-Cys-Cys-Ile
(SEQ ID NO:5);

Gly-Cys-Cys-Ala-Arg-Leu-Thr-Cys-Cys-Val (SEQ ID NO:6);
Asn-Gly-Cys-Cys-Xaa₁-Xaa₅-Gln-Met-Arg-Cys-Cys-Thr (SEQ ID NO:7);

Asp-Xaa₃-Asn-Ser-Cys-Cys-Gly-Xaa₅-Asn-Xaa₁-Gly-Cys-Cys-Xaa₁-Xaa₃ (SEQ ID NO:8);

Xaa₄-Gly-Xaa₃-Cys-Cys-Xaa₅-Xaa₆-Asn-Ile-Arg-Cys-Cys-Val
(SEQ ID NO:9);

Xaa₆-Cys-Cys-Xaa₆-Asp-Gly-Xaa₃-Cys-Cys-Thr-Ala-Ala-Xaa₁-
Leu-Thr (SEQ ID NO:10);

Gly-Cys-Cys-Xaa₆-Asp-Gly-Xaa₃-Cys-Cys-Thr-Ala-Ala-Xaa₁-
Leu-Thr (SEQ ID NO:11);

Asn-Gly-Cys-Cys-Arg-Ala-Gly-Asp-Cys-Cys-Ser-Arg-Phe-Xaa₆-Ile-Xaa₅-Xaa₆-Asn-Asp-Phe (SEQ ID NO:12);

Asn-Ala-Cys-Cys-Ile-Val-Arg-Gln-Cys-Cys (SEQ ID NO:13);

Asn-Gly-Cys-Cys-Arg-Ala-Gly-Asp-Cys-Cys-Ser (SEQ ID NO:14);

Cys-Cys-Xaa₁-Arg-Arg-Leu-Ala-Cys-Cys-Ile-Ile (SEQ ID NO:15);

Cys-Cys-Xaa₁-Asn-Xaa₅-Xaa₁-Cys-Cys-Phe-Ile (SEQ ID NO:16);

Gly-Cys-Cys-Ala-Met-Leu-Thr-Cys-Cys-Val (SEQ ID NO:17);

Leu-Cys-Cys-Val-Thr-Xaa₆-Asp-Xaa₃-Cys-Cys-Xaa₆-Xaa₃-Xaa₃ (SEQ ID NO:18); and

Val-Cys-Cys-Arg-Xaa₁-Val-Gln-Asp-Cys-Cys-Ser (SEQ ID NO:19);

wherein Xaa₁ is Pro or hydroxy-Pro; Xaa₂ is Tyr, mono-halo-Tyr, di-halo-Tyr, O-sulpho-Tyr, O-phospho-Tyr or nitro-Tyr; Xaa₃ is Trp or halo-Trp; Xaa₄ is Gln or pyro-Glu; Xaa₅ is Lys, N-methyl-Lys, N,N-dimethyl-Lys or N,N,N-trimethyl-Lys, Xaa₆ is Glu or gamma-carboxy-Glu (Gla); and the C-terminus contains a carboxyl or amide group. The halo is preferably bromine, chlorine or iodine, more preferably iodine for Tyr and bromine for Trp. In addition, the Arg residues may be substituted by Lys, ornithine, homoarginine, N-methyl-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys or any unnatural basic amino acid; the Lys residues may be substituted by Arg, ornithine, homoarginine, N-methyl-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys or any unnatural basic amino acid; the Tyr residues may be substituted with any unnatural hydroxy containing amino acid; the Ser residues may be substituted with Thr; the Thr residues may be substituted with Ser; and the Phe and Trp residues may be substituted with any unnatural aromatic amino acid. The Cys residues may be in D or L configuration and may optionally be substituted with homocysteine (D or L). The Tyr residues may be substituted with the 3-hydroxyl or 2-hydroxyl isomers and corresponding O-sulpho- and O-phospho-derivatives. The acidic amino acid

residues may be substituted with any synthetic acidic amino acid, e.g., tetrazolyl derivatives of Gly and Ala.

[00016] More specifically, the present invention is directed to the following τ -conotoxin peptides of general formula I:

- AuVA: SEQ ID NO:2, wherein Xaa₁ is Pro, Xaa₂ is Tyr and Xaa₃ is Trp;
- AuVB: SEQ ID NO:3, wherein Xaa₁ is Pro, Xaa₂ is Tyr and Xaa₃ is Trp;
- Tx5.1: SEQ ID NO:4, wherein Xaa₂ is Tyr and Xaa₃ is Trp;
- G5.1: SEQ ID NO:5, wherein Xaa₃ is Trp, Xaa₄ is Gln, Xaa₅ is Lys and Xaa₆ is Glu;
- Qc5.1: SEQ ID NO:6;
- PVA: SEQ ID NO:7, wherein Xaa₁ is Pro and Xaa₅ is Lys;
- Im5.1: SEQ ID NO:8, wherein Xaa₁ is Pro, Xaa₃ is Trp and Xaa₅ is Lys;
- G5.2: SEQ ID NO:9, wherein Xaa₃ is Trp, Xaa₄ is Gln, Xaa₅ is Lys and Xaa₆ is Glu;
- Tx5.2a: SEQ ID NO:10, wherein Xaa₁ is Pro, Xaa₃ is Trp and Xaa₆ is Glu;
- Tx5.2b: SEQ ID NO:11, wherein Xaa₁ is Pro, Xaa₃ is Trp and Xaa₆ is Glu;
- Mr5.1: SEQ ID NO:12, wherein Xaa₅ is Lys and Xaa₆ is Glu;
- Mr5.2: SEQ ID NO:13;
- Mr5.3: SEQ ID NO:14;
- Ca5.1: SEQ ID NO:15, wherein Xaa₁ is Pro;
- Ca5.2: SEQ ID NO:16, wherein Xaa₁ is Pro and Xaa₅ is Lys;
- Qc5.2: SEQ ID NO:17;

Gm5.1: SEQ ID NO:18, wherein Xaa₃ is Trp and Xaa₆ is Glu; and

Gm5.2: SEQ ID NO:19, wherein Xaa₁ is Pro.

The C-terminus preferably contains a carboxyl group for the peptides AuVA, AuVB, G5.1, PVA, G5.2, Mr5.2, Mr5.3 and Gm5.1. The C-terminus of the other peptides preferably contains an amide group.

[00017] Examples of unnatural aromatic amino acid include, but are not limited to, such as nitro-Phe, 4-substituted-Phe wherein the substituent is C₁-C₃ alkyl, carboxyl, hydroxymethyl, sulphomethyl, halo, phenyl, -CHO, -CN, -SO₃H and -NHAc. Examples of unnatural hydroxy containing amino acid, include, but are not limited to, such as 4-hydroxymethyl-Phe, 4-hydroxyphenyl-Gly, 2,6-dimethyl-Tyr and 5-amino-Tyr. Examples of unnatural basic amino acids include, but are not limited to, N-1-(2-pyrazolinyl)-Arg, 2-(4-piperinyl)-Gly, 2-(4-piperinyl)-Ala, 2-[3-(2S)pyrrolininyl]-Gly and 2-[3-(2S)pyrrolininyl]-Ala. These and other unnatural basic amino acids, unnatural hydroxy containing amino acids or unnatural aromatic amino acids are described in Building Block Index, Version 3.0 (1999 Catalog, pages 4-47 for hydroxy containing amino acids and aromatic amino acids and pages 66-87 for basic amino acids; see also <http://www.amino-acids.com>), incorporated herein by reference, by and available from RSP Amino Acid Analogues, Inc., Worcester, MA. Examples of synthetic acid amino acids include those derivatives bearing acidic functionality, including carboxyl, phosphate, sulfonate and synthetic tetrazolyl derivatives such as described by Ornstein et al. (1993) and in U.S. Patent No. 5,331,001, each incorporated herein by reference.

[00018] Optionally, in the peptides of general formula I and the specific peptides described above, the Asn residues may be modified to contain an N-glycan and the Ser and Thr residues may be modified to contain an O-glycan. In accordance with the present invention, a glycan shall mean any N-, S- or O-linked mono-, di-, tri-, poly- or oligosaccharide that can be attached to any hydroxy, amino or thiol group of natural or modified amino acids by synthetic or enzymatic methodologies known in the art. The monosaccharides making up the glycan can include D-allose, D-altrose, D-glucose, D-mannose, D-gulose, D-idose, D-galactose, D-talose, D-galactosamine, D-glucosamine, D-N-acetyl-glucosamine (GlcNAc), D-N-acetyl-galactosamine (GalNAc), D-fucose or D-arabinose. These saccharides may be structurally modified, e.g., with one or more O-sulfate, O-phosphate, O-acetyl or acidic groups, such as sialic acid, including combinations thereof. The glycan may also include similar polyhydroxy groups, such as D-penicillamine 2,5 and halogenated derivatives thereof or polypropylene glycol derivatives. The glycosidic linkage is beta and 1-4 or 1-3, preferably 1-3. The linkage between the glycan and the amino acid may be alpha or beta, preferably alpha and is 1-.

[00019] Core O-glycans have been described by Van de Steen et al. (1998), incorporated herein by reference. Mucin type O-linked oligosaccharides are attached to Ser or Thr (or other hydroxylated residues of the present peptides) by a GalNAc residue. The monosaccharide building blocks and the linkage attached to this first GalNAc residue define the "core glycans," of which eight have been identified. The type of glycosidic linkage (orientation and connectivities) are defined for each core glycan. Suitable glycans and glycan analogs are described further in U.S. Serial No.

09/420,797, filed 19 October 1999 and in PCT Application No. PCT/US99/24380, filed 19 October 1999, both incorporated herein by reference. A preferred glycan is Gal(β 1-3)GalNAc(α 1-).

[00020] Optionally, in the peptides of general formulas I and II and the specific peptides described above, pairs of Cys residues may be replaced pairwise with isosteric lactam or ester-thioether replacements, such as Ser/(Glu or Asp), Lys/(Glu or Asp) or Cys/Ala combinations. Sequential coupling by known methods (Barnay et al., 2000; Hruby et al., 1994; Bitan et al., 1997) allows replacement of native Cys bridges with lactam bridges. Thioether analogs may be readily synthesized using halo-Ala residues commercially available from RSP Amino Acid Analogues.

[00021] The present invention is further directed to propeptides and nucleic acid sequences encoding the propeptides or peptides as described in further detail herein.

SUMMARY OF THE SEQUENCE LISTING

[00022] SEQ ID NO:1 is generic formula I for τ -conotoxin peptides. SEQ ID NO:2 is a generic formula for the peptide AuVA. SEQ ID NO:3 is a generic formula for the peptide AuVB. SEQ ID NO:4 is a generic formula for the peptide Tx5.1. SEQ ID NO:5 is a generic formula for the peptide G5.1. SEQ ID NO:6 is a generic formula for the peptide Qc5.1. SEQ ID NO:7 is a generic formula for the peptide PVA. SEQ ID NO:8 is a generic formula for the peptide Im5.1. SEQ ID NO:9 is a generic sequence for the peptide G5.2. SEQ ID NO:10 is a generic sequence for the peptide Tx5.2a. SEQ ID NO:11 is a generic sequence for the peptide Tx5.2b. SEQ ID NO:12 is a generic sequence for the peptide

Mr5.1. SEQ ID NO:13 is a generic sequence for the peptide
Mr5.2. SEQ ID NO:14 is a generic formula for the peptide
Mr5.3. SEQ ID NO:15 is a generic formula for the peptide
Ca5.1. SEQ ID NO:16 is a generic formula for the peptide
Ca5.2. SEQ ID NO:17 is a generic formula for the peptide
Qc5.2. SEQ ID NO:18 is a generic formula for the peptide
Gm5.1. SEQ ID NO:19 is a generic formula for the peptide
Gm5.2. SEQ ID NO:20 is a DNA sequence coding for the Tx5.1
propeptide. SEQ ID NO:21 is the amino acid sequence of the
Tx5.1 propeptide. SEQ ID NO:22 is a DNA sequence coding for
the G5.1 propeptide. SEQ ID NO:23 is the amino acid
sequence of the G5.1 propeptide. SEQ ID NO:24 is a DNA
sequence coding for the Qc5.1 propeptide. SEQ ID NO:25 is
the amino acid sequence of the Qc5.1 propeptide. SEQ ID
NO:26 is a DNA sequence coding for the Im5.1 propeptide.
SEQ ID NO:27 is the amino acid sequence of the Im5.1
propeptide. SEQ ID NO:28 is a DNA sequence coding for the
G5.2 propeptide. SEQ ID NO:29 is the amino acid sequence of
the G5.2 propeptide. SEQ ID NO:30 is a DNA sequence coding
for the Tx5.2 propeptide. SEQ ID NO:31 is the amino acid
sequence of the Tx5.2 propeptide. SEQ ID NO:32 is a DNA
sequence coding for the Tx5.3 propeptide. SEQ ID NO:33 is
the amino acid sequence of the Tx5.3 propeptide. SEQ ID
NO:34 is a DNA sequence coding for the Mr5.1 peptide. SEQ
ID NO:35 is the amino acid sequence of the Mr5.1 peptide.
SEQ ID NO:36 is a DNA sequence coding for the Mr5.2 peptide.
SEQ ID NO:37 is the amino acid sequence of the Mr5.2
peptide. SEQ ID NO:38 is a DNA sequence coding for the
Mr5.3 propeptide. SEQ ID NO:39 is the amino acid sequence
of the Mr5.3 propeptide. SEQ ID NO:40 is a DNA sequence
coding for the Ca5.1 propeptide. SEQ ID NO:41 is the amino
acid sequence of the Ca5.1 propeptide. SEQ ID NO:42 is a

DNA sequence coding for the Ca5.2 propeptide. SEQ ID NO:43 is the amino acid sequence of the Ca5.2 propeptide. SEQ ID NO:44 is a DNA sequence coding for the Qc5.2 propeptide. SEQ ID NO:45 is the amino acid sequence of the Qc5.2 propeptide. SEQ ID NO:46 is a DNA sequence coding for the Gm5.1 propeptide. SEQ ID NO:47 is the amino acid sequence of the Gm5.1 propeptide. SEQ ID NO:48 is a DNA sequence coding for the Gm5.2 propeptide. SEQ ID NO:49 is the amino acid sequence of the Gm5.2 propeptide.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[00023] The invention relates to relatively short peptides (termed τ -conotoxins herein), about 10-25 residues in length, which are naturally available in minute amounts in the venom of the cone snails or analogous to the naturally available peptides, and which preferably include two disulfide bonds.

[00024] The present invention, in another aspect, relates to a pharmaceutical composition comprising an effective amount of an τ -conotoxin peptide, a mutein thereof, an analog thereof, an active fragment thereof or pharmaceutically acceptable salts. Such a pharmaceutical composition has the capability of acting as an antagonist for acetylcholine receptors and as analgesic agents for the treatment of pain, including migraine. Thus, the pharmaceutical compositions of the present invention are useful in the treatment of pain (whether acute or chronic), including chronic pain, and neuropathic pain, without undesirable side effects.

[00025] The τ -conotoxin peptides described herein are sufficiently small to be chemically synthesized. General chemical syntheses for preparing the foregoing τ -conotoxin

peptides are described hereinafter. Various ones of the τ -conotoxin peptides can also be obtained by isolation and purification from specific *Conus* species using the technique described in U.S. Patent Nos. 4,447,356 (Olivera et al., 1984); 5,514,774; 5,719,264; and 5,591,821, as well as in PCT published application WO 98/03189, the disclosures of which are incorporated herein by reference.

[00026] Although the τ -conotoxin peptides of the present invention can be obtained by purification from cone snails, because the amounts of τ -conotoxin peptides obtainable from individual snails are very small, the desired substantially pure τ -conotoxin peptides are best practically obtained in commercially valuable amounts by chemical synthesis using solid-phase strategy. For example, the yield from a single cone snail may be about 10 micrograms or less of τ -conotoxin peptide. By "substantially pure" is meant that the peptide is present in the substantial absence of other biological molecules of the same type; it is preferably present in an amount of at least about 85% purity and preferably at least about 95% purity. Chemical synthesis of biologically active τ -conotoxin peptides depends of course upon correct determination of the amino acid sequence.

[00027] The τ -conotoxin peptides can also be produced by recombinant DNA techniques well known in the art. Such techniques are described by Sambrook et al. (1989). A gene of interest (i.e., a gene that encodes a suitable τ -conotoxin peptide) can be inserted into a cloning site of a suitable expression vector by using standard techniques. These techniques are well known to those skilled in the art. The expression vector containing the gene of interest may then be used to transfect the desired cell line. Standard transfection techniques such as calcium phosphate

co-precipitation, DEAE-dextran transfection or electroporation may be utilized. A wide variety of host/expression vector combinations may be used to express a gene encoding a conotoxin peptide of interest. Such combinations are well known to a skilled artisan. The peptides produced in this manner are isolated, reduced if necessary, and oxidized to form the correct disulfide bonds.

[00028] One method of forming disulfide bonds in the α -conotoxin peptides of the present invention is the air oxidation of the linear peptides for prolonged periods under cold room temperatures or at room temperature. This procedure results in the creation of a substantial amount of the bioactive, disulfide-linked peptides. The oxidized peptides are fractionated using reverse-phase high performance liquid chromatography (HPLC) or the like, to separate peptides having different linked configurations. Thereafter, either by comparing these fractions with the elution of the native material or by using a simple assay, the particular fraction having the correct linkage for maximum biological potency is easily determined. However, because of the dilution resulting from the presence of other fractions of less biopotency, a somewhat higher dosage may be required.

[00029] The peptides are synthesized by a suitable method, such as by exclusively solid-phase techniques, by partial solid-phase techniques, by fragment condensation or by classical solution couplings.

[00030] In conventional solution phase peptide synthesis, the peptide chain can be prepared by a series of coupling reactions in which constituent amino acids are added to the growing peptide chain in the desired sequence. Use of various coupling reagents, e.g., dicyclohexylcarbodiimide or

diisopropylcarbonyldimidazole, various active esters, e.g., esters of N-hydroxyphthalimide or N-hydroxy-succinimide, and the various cleavage reagents, to carry out reaction in solution, with subsequent isolation and purification of intermediates, is well known classical peptide methodology. Classical solution synthesis is described in detail in the treatise, "Methoden der Organischen Chemie (Houben-Weyl): Synthese von Peptiden," (1974). Techniques of exclusively solid-phase synthesis are set forth in the textbook, "Solid-Phase Peptide Synthesis," (Stewart and Young, 1969), and are exemplified by the disclosure of U.S. Patent 4,105,603 (Vale et al., 1978). The fragment condensation method of synthesis is exemplified in U.S. Patent 3,972,859 (1976). Other available syntheses are exemplified by U.S. Patents No. 3,842,067 (1974) and 3,862,925 (1975). The synthesis of peptides containing γ -carboxyglutamic acid residues is exemplified by Rivier et al. (1987), Nishiuchi et al. (1993) and Zhou et al. (1996).

[00031] Common to such chemical syntheses is the protection of the labile side chain groups of the various amino acid moieties with suitable protecting groups which will prevent a chemical reaction from occurring at that site until the group is ultimately removed. Usually also common is the protection of an α -amino group on an amino acid or a fragment while that entity reacts at the carboxyl group, followed by the selective removal of the α -amino protecting group to allow subsequent reaction to take place at that location. Accordingly, it is common that, as a step in such a synthesis, an intermediate compound is produced which includes each of the amino acid residues located in its desired sequence in the peptide chain with appropriate side-

chain protecting groups linked to various ones of the residues having labile side chains.

[00032] As far as the selection of a side chain amino protecting group is concerned, generally one is chosen which is not removed during deprotection of the α -amino groups during the synthesis. However, for some amino acids, e.g., His, protection is not generally necessary. In selecting a particular side chain protecting group to be used in the synthesis of the peptides, the following general rules are followed: (a) the protecting group preferably retains its protecting properties and is not split off under coupling conditions, (b) the protecting group should be stable under the reaction conditions selected for removing the α -amino protecting group at each step of the synthesis, and (c) the side chain protecting group must be removable, upon the completion of the synthesis containing the desired amino acid sequence, under reaction conditions that will not undesirably alter the peptide chain.

[00033] It should be possible to prepare many, or even all, of these peptides using recombinant DNA technology. However, when peptides are not so prepared, they are preferably prepared using the Merrifield solid-phase synthesis, although other equivalent chemical syntheses known in the art can also be used as previously mentioned. Solid-phase synthesis is commenced from the C-terminus of the peptide by coupling a protected α -amino acid to a suitable resin. Such a starting material can be prepared by attaching an α -amino-protected amino acid by an ester linkage to a chloromethylated resin or a hydroxymethyl resin, or by an amide bond to a benzhydrylamine (BHA) resin or paramethylbenzhydrylamine (MBHA) resin. Preparation of the hydroxymethyl resin is described by Bodansky et al.

(1966). Chloromethylated resins are commercially available from Bio Rad Laboratories (Richmond, CA) and from Lab. Systems, Inc. The preparation of such a resin is described by Stewart and Young (1969). BHA and MBHA resin supports are commercially available, and are generally used when the desired polypeptide being synthesized has an unsubstituted amide at the C-terminus. Thus, solid resin supports may be any of those known in the art, such as one having the formulae $-O-CH_2$ -resin support, $-NH$ BHA resin support, or $-NH$ -MBHA resin support. When the unsubstituted amide is desired, use of a BHA or MBHA resin is preferred, because cleavage directly gives the amide. In case the N-methyl amide is desired, it can be generated from an N-methyl BHA resin. Should other substituted amides be desired, the teaching of U.S. Patent No. 4,569,967 (Kornreich et al., 1986) can be used, or should still other groups than the free acid be desired at the C-terminus, it may be preferable to synthesize the peptide using classical methods as set forth in the Houben-Weyl text (1974).

[00034] The C-terminal amino acid, protected by Boc or Fmoc and by a side-chain protecting group, if appropriate, can be first coupled to a chloromethylated resin according to the procedure set forth in K. Horiki et al. (1978), using KF in DMF at about 60°C for 24 hours with stirring, when a peptide having free acid at the C-terminus is to be synthesized. Following the coupling of the BOC-protected amino acid to the resin support, the α -amino protecting group is removed, as by using trifluoroacetic acid (TFA) in methylene chloride or TFA alone. The deprotection is carried out at a temperature between about 0°C and room temperature. Other standard cleaving reagents, such as HCl in dioxane, and conditions for removal of specific α -amino

protecting groups may be used as described in Schroder & Lubke (1965).

[00035] After removal of the α -amino-protecting group, the remaining α -amino- and side chain-protected amino acids are coupled step-wise in the desired order to obtain the intermediate compound defined hereinbefore, or as an alternative to adding each amino acid separately in the synthesis, some of them may be coupled to one another prior to addition to the solid phase reactor. Selection of an appropriate coupling reagent is within the skill of the art. Particularly suitable as a coupling reagent is N,N'-dicyclohexylcarbodiimide (DCC, DIC, HBTU, HATU, TBTU in the presence of HoBt or HoAt).

[00036] The activating reagents used in the solid phase synthesis of the peptides are well known in the peptide art. Examples of suitable activating reagents are carbodiimides, such as N,N'-diisopropylcarbodiimide and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide. Other activating reagents and their use in peptide coupling are described by Schroder & Lubke (1965) and Kapoor (1970).

[00037] Each protected amino acid or amino acid sequence is introduced into the solid-phase reactor in about a twofold or more excess, and the coupling may be carried out in a medium of dimethylformamide (DMF):CH₂Cl₂ (1:1) or in DMF or CH₂Cl₂ alone. In cases where intermediate coupling occurs, the coupling procedure is repeated before removal of the α -amino protecting group prior to the coupling of the next amino acid. The success of the coupling reaction at each stage of the synthesis, if performed manually, is preferably monitored by the ninhydrin reaction, as described by Kaiser et al. (1970). Coupling reactions can be performed automatically, as on a Beckman 990 automatic synthesizer,

using a program such as that reported in Rivier et al. (1978).

[00038] After the desired amino acid sequence has been completed, the intermediate peptide can be removed from the resin support by treatment with a reagent, such as liquid hydrogen fluoride or TFA (if using Fmoc chemistry), which not only cleaves the peptide from the resin but also cleaves all remaining side chain protecting groups and also the α -amino protecting group at the N-terminus if it was not previously removed to obtain the peptide in the form of the free acid. If Met is present in the sequence, the Boc protecting group is preferably first removed using trifluoroacetic acid (TFA)/ethanedithiol prior to cleaving the peptide from the resin with HF to eliminate potential S-alkylation. When using hydrogen fluoride or TFA for cleaving, one or more scavengers such as anisole, cresol, dimethyl sulfide and methylethyl sulfide are included in the reaction vessel.

[00039] Cyclization of the linear peptide is preferably affected, as opposed to cyclizing the peptide while a part of the peptido-resin, to create bonds between Cys residues. To effect such a disulfide cyclizing linkage, fully protected peptide can be cleaved from a hydroxymethylated resin or a chloromethylated resin support by ammonolysis, as is well known in the art, to yield the fully protected amide intermediate, which is thereafter suitably cyclized and deprotected. Alternatively, deprotection, as well as cleavage of the peptide from the above resins or a benzhydrylamine (BHA) resin or a methylbenzhydrylamine (MBHA), can take place at 0°C with hydrofluoric acid (HF) or TFA, followed by oxidation as described above.

[00040] The peptides are also synthesized using an automatic synthesizer. Amino acids are sequentially coupled to an MBHA Rink resin (typically 100 mg of resin) beginning at the C-terminus using an Advanced Chemtech 357 Automatic Peptide Synthesizer. Couplings are carried out using 1,3-diisopropylcarbodiimide in N-methylpyrrolidinone (NMP) or by 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and diethylisopropylethylamine (DIEA). The Fmoc protecting group is removed by treatment with a 20% solution of piperidine in dimethylformamide (DMF). Resins are subsequently washed with DMF (twice), followed by methanol and NMP.

[00041] Muteins, analogs or active fragments, of the foregoing conotoxin peptides are also contemplated here. See, e.g., Hammerland et al, Eur. J. Pharmacol., 226, pp. 239-244 (1992). Derivative muteins, analogs or active fragments of the conotoxin peptides may be synthesized according to known techniques, including conservative amino acid substitutions, such as outlined in U.S. Pat. Nos. 5,545,723 (see particularly col. 2, line 50--col. 3, line 8); 5,534,615 (see particularly col. 19, line 45--col. 22, line 33); and 5,364,769 (see particularly col. 4, line 55--col. 7, line 26), each herein incorporated by reference.

[00042] Pharmaceutical compositions containing a compound of the present invention or its pharmaceutically acceptable salts as the active ingredient can be prepared according to conventional pharmaceutical compounding techniques. See, for example, *Remington's Pharmaceutical Sciences*, 18th Ed. (1990, Mack Publishing Co., Easton, PA). Typically, an antagonistic amount of the active ingredient will be admixed with a pharmaceutically acceptable carrier. The carrier may

take a wide variety of forms depending on the form of preparation desired for administration, e.g., intravenous, oral or parenteral. The compositions may further contain antioxidizing agents, stabilizing agents, preservatives and the like. For examples of delivery methods see U.S. Patent No. 5,844,077, incorporated herein by reference.

[00043] For oral administration, the compounds can be formulated into solid or liquid preparations such as capsules, pills, tablets, lozenges, melts, powders, suspensions or emulsions. In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, suspending agents, and the like in the case of oral liquid preparations (such as, for example, suspensions, elixirs and solutions); or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations (such as, for example, powders, capsules and tablets). Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar-coated or enteric-coated by standard techniques. The active agent can be encapsulated to make it stable to passage through the gastrointestinal tract while at the same time allowing for passage across the blood brain barrier. See for example, WO 96/11698.

[00044] For parenteral administration, the compound may be dissolved in a pharmaceutical carrier and administered as either a solution or a suspension. Illustrative of suitable carriers are water, saline, dextrose solutions, fructose

solutions, ethanol, or oils of animal, vegetative or synthetic origin. The carrier may also contain other ingredients, for example, preservatives, suspending agents, solubilizing agents, buffers and the like. When the compounds are being administered intrathecally, they may also be dissolved in cerebrospinal fluid.

[00045] The active agent is preferably administered in an therapeutically effective amount. The actual amount administered, and the rate and time-course of administration, will depend on the nature and severity of the condition being treated. Prescription of treatment, e.g. decisions on dosage, timing, etc., is within the responsibility of general practitioners or specialists, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of techniques and protocols can be found in *Remington's Pharmaceutical Sciences*. Typically the active agents of the present invention exhibit their effect at a dosage range from about 0.001 mg/kg to about 250 mg/kg, preferably from about 0.01 mg/kg to about 100 mg/kg of the active ingredient, more preferably from about 0.05 mg/kg to about 75 mg/kg. A suitable dose can be administered in multiple sub-doses per day. Typically, a dose or sub-dose may contain from about 0.1 mg to about 500 mg of the active ingredient per unit dosage form. A more preferred dosage will contain from about 0.5 mg to about 100 mg of active ingredient per unit dosage form. Dosages are generally initiated at lower levels and increased until desired effects are achieved.

[00046] Alternatively, targeting therapies may be used to deliver the active agent more specifically to certain types of cell, by the use of targeting systems such as antibodies or cell specific ligands. Targeting may be desirable for a variety of reasons, e.g. if the agent is unacceptably toxic, or if it would otherwise require too high a dosage, or if it would not otherwise be able to enter the target cells.

[00047] The active agents, which are peptides, can also be administered in a cell based delivery system in which a DNA sequence encoding an active agent is introduced into cells designed for implantation in the body of the patient, especially in the spinal cord region. Suitable delivery systems are described in U.S. Patent No. 5,550,050 and published PCT Application Nos. WO 92/19195, WO 94/25503, WO 95/01203, WO 95/05452, WO 96/02286, WO 96/02646, WO 96/40871, WO 96/40959 and WO 97/12635. Suitable DNA sequences can be prepared synthetically for each active agent on the basis of the developed sequences and the known genetic code.

EXAMPLES

[00048] The present invention is described by reference to the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below were utilized.

EXAMPLE 1

Isolation of α -Conotoxins

[00049] Crude venom was extracted from venom ducts (Cruz et al., 1976), and the components were purified as previously

described (Cartier et al., 1996). The crude extract from venom ducts was purified by reverse phase liquid chromatography (RPLC) using a Vydac C₁₈ semi-preparative column (10 x 250 mm). Further purification of bioactive peaks was done on a Vydac C₁₈ analytical column (4.6 x 220 mm). The effluents were monitored at 220 nm. Peaks were collected, and aliquots were assayed for activity.

[00050] The amino acid sequence of the purified peptides were determined by standard methods. The purified peptides were reduced and alkylated prior to sequencing by automated Edman degradation on an Applied Biosystems 477A Protein Sequencer with a 120A Analyzer (DNA/Peptide Facility, University of Utah) (Martinez et al., 1995; Shon et al., 1994).

[00051] In accordance with this method, peptides AuVA, AuVB and PVA were obtained.

EXAMPLE 2

Synthesis of Conopeptides

[00052] The synthesis of conopeptides, either the mature toxins or the precursor peptides, was separately performed using conventional protection chemistry as described by Cartier et al. (1996). Briefly, the linear chains were built on Rink amide resin by Fmoc procedures with 2-(1H-benzotriol-1-yl)-1,1,3,3,-tetramethyluronium tetrafluoroborated coupling using an ABI model 430A peptide synthesizer with amino acid derivatives purchased from Bachem (Torrence CA). Orthogonal protection was used on cysteines: two cysteines were protected as the stable Cys(S-acetamidomethyl), while the other two cysteines were protected as the acid-labile Cys(S-trityl). After removal of the terminal Fmoc protecting group and cleavage of the peptides from the resins, the released

peptides were precipitated by filtering the reaction mixture into -10°C methyl t-butyl ether, which removed the protecting groups except the Cys(S-acetamidomethyl). The peptides were dissolved in 0.1% TFA and 60% acetonitrile and purified by RPLC on a Vydac C₁₈ preparative column (22 x 250 mm) and eluted at a flow rate of 20 mL/min with a gradient of acetonitrile in 0.1% TFA.

[00053] The disulfide bridges in the three conopeptides were formed as described in Cartier et al. (1996). Briefly, the disulfide bridges between one pair of cysteines were formed by air oxidation which was judged to be complete by analytical RPLC. The monocyclic peptides were purified by RPLC on a Vydac C₁₈ preparative column (22 x 250 mm) and eluted with a gradient of acetonitrile in 0.1% TFA. Removal of S-acetamidomethyl groups and closure of the disulfide bridge between the other pair of cysteines was carried out simultaneously by iodine oxidation. The cyclic peptides were purified by RPLC on a Vydac C₁₈ preparative column (22 x 250 mm) and eluted with a gradient of acetonitrile in 0.1% TFA.

EXAMPLE 3

Isolation of DNA Encoding τ -Conotoxins

[00054] DNA coding for τ -conotoxins was isolated and cloned in accordance with conventional techniques using general procedures well known in the art, such as described in Olivera et al. (1996). Alternatively, cDNA libraries were prepared from *Conus* venom duct using conventional techniques. DNA from single clones was amplified by conventional techniques using primers which correspond approximately to the M13 universal priming site and the M13 reverse universal priming site. Clones having a size of approximately 300-500 nucleotides were sequenced and screened for similarity in sequence to known τ -

conotoxins isolated in Example 1. The DNA sequences and encoded propeptide sequences are set forth in Tables 1-15. DNA sequences coding for the mature toxin can also be prepared on the basis of the DNA sequences set forth in these Tables.

TABLE 1

DNA Sequence (SEQ ID NO:20) and Protein Sequence (SEQ ID NO:21) of Tx5.1

gg tact caac ga actt caag ac acatt ctt ttc acct gga cac gga agc tg acta caag

caga atg tgc tgt ctc cca gtg ttc gtc att ctt ctg ctg ctg att gca
Met Cys Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala

tct gca cct agc gtt gat gcc caa ccg aag acc aaa gat gat gtg ccc
Ser Ala Pro Ser Val Asp Ala Gln Pro Lys Thr Lys Asp Asp Val Pro

ctg gca cct ttg cac gat aat gca aag agt gca cta caa cat ttg aac
Leu Ala Pro Leu His Asp Asn Ala Lys Ser Ala Leu Gln His Leu Asn

caa cgc tgc tgc caa aca ttc tat tgg tgc tgt gtt caa ggg aaa
Gln Arg Cys Cys Gln Thr Phe Tyr Trp Cys Cys Val Gln Gly Lys

tgaattt gga tg agacc cct gc gaact gtc cat ggat gtg ag attt ggaa agc agact gt

tcctttc gca cgtgttc gtg gaatttt gaa tggtc gttaa caacacgctg ccacttgcaa

gctactatct ctctgtcctt tcatctgtgg aactggatga cctaacaact gaaatatcat

agaaattttt cagtgggtat acactatgac catgtagtca gtaattacat catttggacc

ttttgaaata tttttcaaaa tgttaagatt tttccccng gaaaggnctt ttgaagtaaa

tatt

TABLE 2

DNA Sequence (SEQ ID NO:22) and Protein Sequence (SEQ ID NO:23) of G5.1

```

atg tgc tgt ctc cca gtc ttc gtc att ctt ctg ttg ctg att aca tct
Met Cys Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Thr Ser

gca cct agc gtt gat gct cta ccg aag acc agg gat gat gtg ccc cta
Ala Pro Ser Val Asp Ala Leu Pro Lys Thr Arg Asp Asp Val Pro Leu

gca tct ttc cac ggt gga tat aat gca agg aga atc cta caa agg cgt
Ala Ser Phe His Gly Gly Tyr Asn Ala Arg Arg Ile Leu Gln Arg Arg

cag ggc tgg tgc tgc aaa gaa aat att gcg tgc tgt ata tagtggtaac
Gln Gly Trp Cys Cys Lys Glu Asn Ile Ala Cys Cys Ile

gggaaatgac tttggatgag acccctgcaa actgtccctg gatgtgaaat ttggaaagta

gactgttcct ttcgcgcgtg ttcgtggaat ttcaaattgt cgtcaacaac aactgctac

ttgcaaagct actatctctc tgtcctttca tctgtggaac tgggtgatct aacagctgaa

atgtcgcaga aatTTTTTcaa ttggtctata ctatgaccat gta

```

TABLE 3

DNA Sequence (SEQ ID NO:24) and Protein Sequence (SEQ ID NO:25) of Qc5.1

```

atg cgc tgt gtc cca gtc ttc atc att ctt ctg ctg ctg agt cca tct
Met Arg Cys Val Pro Val Phe Ile Ile Leu Leu Leu Leu Ser Pro Ser

gca cct agc gtt gat gcc cat ccg atg acc aaa gat gat gtg ccc cag
Ala Pro Ser Val Asp Ala His Pro Met Thr Lys Asp Asp Val Pro Gln

gca tca ttc cat gat gat gca aag cga acc cta caa gta cct tgg atg
Ala Ser Phe His Asp Asp Ala Lys Arg Thr Leu Gln Val Pro Trp Met

aaa cgc ggg tgc tgc gca agg ttg act tgc tgc gtt gga cga

```

Lys Arg Gly Cys Cys Ala Arg Leu Thr Cys Cys Val Gly Arg

taaagggaaa tgactttgga tgagaccct gcgaactgtc cctggatgtg aaatttggac
 agcagactgc tcctttcgca cgtgttcgtg gaattttgaa tggtcgttaa caacacgtg
 ccacttgcaa gctattatct ctctgtccct ttatctgtgg aactggataa tctaacaact
 gaaatgtcat tgaaaatttt caatggatat atattatgat ccatata

TABLE 4

DNA Sequence (SEQ ID NO:26) and Protein Sequence (SEQ ID NO:27) of Im5.1

aattcggaag ctgactacaa gcaga atg tac tgt ctc cca gtc ttc atc att
 Met Tyr Cys Leu Pro Val Phe Ile Ile

ctt ctg ctg ctg att tca tct gca cct agc act cct ccc caa cca agg
 Leu Leu Leu Leu Ile Ser Ser Ala Pro Ser Thr Pro Pro Gln Pro Arg

aac aaa gat cgt gtg cac ctg ata tct tta ctc gat aat cac aag caa
 Asn Lys Asp Arg Val His Leu Ile Ser Leu Leu Asp Asn His Lys Gln

atc cta caa aga gat tgg aac agt tgc tgt ggg aaa aat cct ggt tgc
 Ile Leu Gln Arg Asp Trp Asn Ser Cys Cys Gly Lys Asn Pro Gly Cys

tgt cct tgg gga aaa tgactttgga tgagaccct gcaaactgtc cctggatgtg
 Cys Pro Trp Gly Lys

agatttggaa agcagaccgt ttgtggaatt ttgaatggc gttaacaaca cgctgccact

tgcaagctac aatctctctg tcctttcatc ttggaactg gatgatcaaa caactgaaat

gtcatagaaa tttttcaatg ggtatacaat atgtgggcat ttagtcagta attacatcat

ttgg

TABLE 5

DNA Sequence (SEQ ID NO:28) and Protein Sequence (SEQ ID NO:29) of G5.2

```

atg tgc tgt ctc cca gtc ttc gtc att ctt ctg ttg ctg att aca tct
Met Cys Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Thr Ser

gca cct agc gtt gat gct cta ccg aag acc agg gat gat gtg ccc cta
Ala Pro Ser Val Asp Ala Leu Pro Lys Thr Arg Asp Asp Val Pro Leu

gca tct ttc cac ggt gga tat aat gca agg aga atc cta caa agg cgt
Ala Ser Phe His Gly Gly Tyr Asn Ala Arg Arg Ile Leu Gln Arg Arg

cag ggc tgg tgc tgc aaa gaa aat att gcg tgc tgt gta tagtggtaac
Gln Gly Trp Cys Cys Lys Glu Asn Ile Ala Cys Cys Val

gggaaatgac tttggatgag acccctgcaa actgtccctg gatgtgaaat ttggaaagta

gactgttctt ttcgcgcgtg ttcgtggaat ttcaaattggt cgtcaacaac aactgctac

ttgcaaagct actatctctc tgtcctttca tctgtggaac tgggtgatct aacagctgaa

atgtcgcaga aattttttcaa ttggtctata ctatgaccat gtagtcag

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TABLE 6

DNA Sequence (SEQ ID NO:30) and Protein Sequence (SEQ ID NO:31) of Tx5.2a

```

atg cgc tgt ttc cca gtc ttc atc att ctt ctg ctg cta att gca tct
Met Arg Cys Phe Pro Val Phe Ile Ile Leu Leu Leu Leu Ile Ala Ser

gca cct tgc ttt gat gcc cga acg aag acc gat gat gat gtg ccc ctg
Ala Pro Cys Phe Asp Ala Arg Thr Lys Thr Asp Asp Asp Val Pro Leu

tca tct ctc cgc gat aat cta aag cga acg ata cga aca cgc ctg aac
Ser Ser Leu Arg Asp Asn Leu Lys Arg Thr Ile Arg Thr Arg Leu Asn

ata cgc gag tgc tgc gag gat gga tgg tgc tgt act gct gca ccc tta

```

Ile Arg Glu Cys Cys Glu Asp Gly Trp Cys Cys Thr Ala Ala Pro Leu
 aca ggt cgt tagggataaa ggaaaatggc tttggatgag acccctgca
 Thr Gly Arg
 attgtccctg gatgtgagat ttggaaagca gactgttcct ttcgcacgtg ttcgtggaat
 ttcgaatggc cgtaacaac acgctgccac ttgcaagcca ccattctctct gtcctttcgt
 atgtggaact gatatgacta acaactgaaa tgcagaaaag ttttcagtgg gtatacacta
 tgatcgata

TABLE 7

DNA Sequence (SEQ ID NO:32) and Protein Sequence (SEQ ID NO:33) of Tx5.2b

atg cgc tgt ttc cca gtc ttc atc att ctt ctg ttg cta att gca tct
 Met Arg Cys Phe Pro Val Phe Ile Ile Leu Leu Leu Leu Ile Ala Ser
 gca cct tgc ttt gat gcc cga acg aag acc gat gat gat gtg ccc ctg
 Ala Pro Cys Phe Asp Ala Arg Thr Lys Thr Asp Asp Asp Val Pro Leu
 tca tct ctc cgc gat aat cta aag cga acg ata cga aca cgc ctg aac
 Ser Ser Leu Arg Asp Asn Leu Lys Arg Thr Ile Arg Thr Arg Leu Asn
 ata cgc ggg tgc tgc gag gat gga tgg tgc tgt act gct gca ccc tta
 Ile Arg Gly Cys Cys Glu Asp Gly Trp Cys Cys Thr Ala Ala Pro Leu
 aca ggt cgt tagggataaa ggaaaatggc tttggatgag acccctgcaa
 Thr Gly Arg
 attgtccctg gatgtgagat ttggaaagca gactgttcct ttcgcacgtg ttcgtggaat
 ttcgaatggc cgtaacaac acgctgccac ttgcaagcca ccattctctct gtcctttcgt
 atgtggaact gatatgacta acaactgaaa tgcagaaaag ttttcagtgg gtatacacta
 tgatcgata gtcagtaatt

TABLE 8

DNA Sequence (SEQ ID NO:34) and Protein Sequence (SEQ ID NO:35) of Mr5.1

atg cgc tgc ctc cca gtc ttc gtc att ctt ctg ctg ctg att gca tct
Met Arg Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala Ser

gca cct agc gtt gat gcc cga ccg aag acc aaa gat gat atg ccc ctg
Ala Pro Ser Val Asp Ala Arg Pro Lys Thr Lys Asp Asp Met Pro Leu

gca tct ttc cat gat aat gca aag cga atc ctg caa ata ctt cag gac
Ala Ser Phe His Asp Asn Ala Lys Arg Ile Leu Gln Ile Leu Gln Asp

aga aat ggt tgc tgc aga gca gga gac tgc tgt tca cga ttt gag ata
Arg Asn Gly Cys Cys Arg Ala Gly Asp Cys Cys Ser Arg Phe Glu Ile

aag gaa aat gac ttt gga tgagacccct gcaaactgtc cttggatgtg
Lys Glu Asn Asp Phe Gly

agatttggaa agcagactgt tccttttcgca cgtgttcgtg gaatttcgaa tggtcgttaa

caacacgctg ccacttgcaa gctactatct ctctgtcctt ttgtctgtgg aactgtatga

tcaaacaact gaaatgtcat agaaattttt cagtgggtaa acactatgac catgta

TABLE 9

DNA Sequence (SEQ ID NO:36) and Protein Sequence (SEQ ID NO:37) of Mr5.2

ga atg cgc tgc ctc cca gtc ttc gtc att ctt ctg ctg ctg att gca
Met Arg Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala

tct gca cct agc gtt gat gcc cga ccg aag acc aaa gat gat atg ccc
Ser Ala Pro Ser Val Asp Ala Arg Pro Lys Thr Lys Asp Asp Met Pro

ctg gca tct ttc cac gat aat gca aag cga atc ctg caa ata ctt cag
Leu Ala Ser Phe His Asp Asn Ala Lys Arg Ile Leu Gln Ile Leu Gln

gac aga aat gct tgc tgc ata gta agg cag tgc tgt tgatgatttg
 Asp Arg Asn Ala Cys Cys Ile Val Arg Gln Cys Cys

agataaagga aaatgacttt ggatgagacc cctgcaaact gtccctggat gtgagatttg
 gaaagcagac tgttcctttc gcacgtgttc gtggaatttc gaatggctgt taacaacacg
 ctgccacttg caagctacta tctctctgtc ctttcatctg tggaactgta tgatcaaaca
 actgaaatgt catagaaatt tttcagtggg taaacactat gatcatgtag tcagtaatta
 catcatttgg aattccatca agcttatcga taccgtcgac ctcgaggggg ggcccggg

TABLE 10

DNA Sequence (SEQ ID NO:38) and Protein Sequence (SEQ ID NO:39) of Mr5.3

atg cgc tgc ctc cca gtc ttt gtc att ctt ctg ctg ctg att gca tct
 Met Arg Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala Ser

gca cct agc gtt gat gcc cga ccg aag acc aaa gat gat atg ccc ctg
 Ala Pro Ser Val Asp Ala Arg Pro Lys Thr Lys Asp Asp Met Pro Leu

gca tct ttc cat gat aat gca aag cga atc ctg caa ata ctt cag gac
 Ala Ser Phe His Asp Asn Ala Lys Arg Ile Leu Gln Ile Leu Gln Asp

aga aat ggt tgc tgc aga gca gga gac tgc tgt tca tgatttgaga
 Arg Asn Gly Cys Cys Arg Ala Gly Asp Cys Cys Ser

taaagggaaa tgactttgga tgagaccctt gcaaactgtc cttggatgtg agatttgga

agcagactgt tcctttcgca cgtgttcgtg gaatttcgaa tggtcgttaa caacacgtg

ccacttgcaa gctactatct ctctgtcctt tcctctgtgg aactgtatga tcaaacaact

TABLE 11

DNA Sequence (SEQ ID NO:40) and Protein Sequence (SEQ ID NO:41) of Ca5.1

```

atg cgc tgt ctc ccg gtc ttc atc att ctt ctg ctg ctg att gca tct
Met Arg Cys Leu Pro Val Phe Ile Ile Leu Leu Leu Leu Ile Ala Ser

gca cct ggc gtt gat gcc caa ccg aag acc aaa tat aat gcg ccc ctg
Ala Pro Gly Val Asp Ala Gln Pro Lys Thr Lys Tyr Asn Ala Pro Leu

aca tct ctc cac gat aat gca aag ggt ata cta caa gaa cat tgg aac
Thr Ser Leu His Asp Asn Ala Lys Gly Ile Leu Gln Glu His Trp Asn

aaa cgc tgc tgc ccc aga agg ctt gcc tgc tgt att ata gga cgg aaa
Lys Arg Cys Cys Pro Arg Arg Leu Ala Cys Cys Ile Ile Gly Arg Lys

tgaatgattt tgggtgagat ccctgcaaac tgccctgga tttgaatttt ggaaagcaga

ctgttccttt cgcacgtgtt cgtggaattt cgaatgggtcg ttaacaacac gctgccactt

gcaagctact atctctctgt cctttttctc tgtgaaactg gatgggtctaa caactgaaat

gtcatagaaa attttcaatg ggtatactct atgaccatct a

```

TABLE 12

DNA Sequence (SEQ ID NO:42) and Protein Sequence (SEQ ID NO:43) of Ca5.2

```

atg cgc tgt ctc cca gtc ttc atc att ctt ctg ctg ctg att gca tct
Met Arg Cys Leu Pro Val Phe Ile Ile Leu Leu Leu Leu Ile Ala Ser

gca cct ggc gtt gat gcc caa ccg aag acc aaa tat gat gcg ccc ctg
Ala Pro Gly Val Asp Ala Gln Pro Lys Thr Lys Tyr Asp Ala Pro Leu

aca tct ctc cac gat aat gca aag ggt ata cta caa gaa cat tgg aac
Thr Ser Leu His Asp Asn Ala Lys Gly Ile Leu Gln Glu His Trp Asn

aaa cgc tgc tgc ccc aac aag cct tgc tgt ttt ata gga agg aaa

```

Lys Arg Cys Cys Pro Asn Lys Pro Cys Cys Phe Ile Gly Arg Lys
 tgaatgattt tgggtgagac cctgcaaac tgccttgga tttgaatttt ggaaagcaga
 ctgttccttt cgcacgtgtt cgtggaattt cgaatgggtcg ttaacaacac gctgccactt
 gcaagctact atctctctgt cctttttctc tgtgaaactg gatgggtctaa caactgagat
 gtcatagaaa attttcaatc ggtgtactct atgaccatct a

TABLE 13

DNA Sequence (SEQ ID NO:44) and Protein Sequence (SEQ ID NO:45) of Qc5.2

atg cgc tgt gtc cca gtc ttc atc att ctt ctg ctg ctg agt cca tct
 Met Arg Cys Val Pro Val Phe Ile Ile Leu Leu Leu Leu Ser Pro Ser
 gca cct agc gtt gat gcc cat ccg atg acc aaa gat gat gta ccc cag
 Ala Pro Ser Val Asp Ala His Pro Met Thr Lys Asp Asp Val Pro Gln
 gca tct ctc cat gat gat gca aag cga acc cta caa gta cct tgg atg
 Ala Ser Leu His Asp Asp Ala Lys Arg Thr Leu Gln Val Pro Trp Met
 aaa cgc ggg tgc tgc gca atg ttg act tgc tgc gtt gga cga
 Lys Arg Gly Cys Cys Ala Met Leu Thr Cys Cys Val Gly Arg
 taaagggaaa tgactttgga tgagaccct acgaactgtc cctggatgtg aaatttggac
 agcagactgc tcctttcgca cgtgttcgtg gaatttcgaa tggtcgttaa caacacgctg
 ccacttgcaa gctattatct ctctgtccct ttatctgtgg aactggataa tctaacaact
 gaaacgtcat tgaaaatttt caatggatat atattatgat ccatata

TABLE 14

DNA Sequence (SEQ ID NO:46) and Protein Sequence (SEQ ID NO:47) of Gm5.1

gggcaggtac tcaacgaact tcaggacaca ttcttttcac ctggacacgg gaaactgact

ataagcaga atg cgc tac cta cca gtc ttc gtc att ctt ctg ctg ctg att
Met Arg Tyr Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile

gca tct ata cct agc gat act gtc caa ctg aag acc aaa gat gat atg
Ala Ser Ile Pro Ser Asp Thr Val Gln Leu Lys Thr Lys Asp Asp Met

ccc ctg gca tct ttc cac ggt aat gga aga cga atc ctg cga atg ctt
Pro Leu Ala Ser Phe His Gly Asn Gly Arg Arg Ile Leu Arg Met Leu

tca aac aaa cgc tta tgc tgt gtc acc gag gat tgg tgc tgt gaa tgg
Ser Asn Lys Arg Leu Cys Cys Val Thr Glu Asp Trp Cys Cys Glu Trp

tgg taaaggaaaa tgactttgga tgagaccct gcaaactggt tctggatgtg
Trp

agatttggaa agcagactgt tctttcgcac gtattcgtga aatttcgaat ggctcgtaac

aacacgctgc cacttgcaag ctgctatctc tctgtctttt catctgtgga actgtatgat

ctaacaactg aaatgtcata gacatttttc attgggtata cactatgacc atgtagccag

taattacatc atttggacct tttggatatt ttccagtatg taagtgtggt cccttaaaaa

gtcctttgta attatgtatt ttaanaattt angttttgca cataaattgt aaaacgctgt

cctttctgtt gntcctacat cantgggtggg gaaaagnaaa atgtttggcc ntgggtcaaat

ttaaataatn accctgccgt ttnaatgcng ttattantgg tatttttnaac ntgnacggt

taaactt

TABLE 15

DNA Sequence (SEQ ID NO:48) and Protein Sequence (SEQ ID NO:49) of Gm5.2

ga atg cgc tgt ctc cca gtc ttc gtc att ctt ctg ctg ctg att gca
Met Arg Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala

tct gca cct agc gtt gat gcc caa ccg aag acc aaa gat gat gtg ccc
Ser Ala Pro Ser Val Asp Ala Gln Pro Lys Thr Lys Asp Asp Val Pro

ctg gca cct ttg cac gat aat ata agg agt act cta caa aca ctt cgg
Leu Ala Pro Leu His Asp Asn Ile Arg Ser Thr Leu Gln Thr Leu Arg

aag aaa gtc tgc tgc cgc cca gtg cag gat tgc tgt tca ggg aaa
Lys Lys Val Cys Cys Arg Pro Val Gln Asp Cys Cys Ser Gly Lys

tgaagggaaa tgaatttgga tgagaccct gcgaactgtc cctggatgtg agatttgga

agcagactgt tcctttcgca cgtgttcgtg gaatttcgaa tggtcgttaa caacacgtg

ccacttgcaa gctactatct ctctgtcctt tcactctgcg aactggatga cctaaagctt

gtgata

EXAMPLE 4

Biological Activity of τ -Conotoxins

[00055] The biological activity of τ -conotoxin peptides at the acetylcholine receptor was tested in the fluorescence assay as described by Cornell-Bell et al. (1999). Briefly, primary cortical cells are exposed to acetylcholine in the presence or absence of a τ -conotoxin peptide. Acetylcholine causes the primary cortical cells to flux calcium, which is measured by increases in fluorescence in cells loaded with Fluo-3, a calcium imaging dye. The τ -conotoxin peptide AuVA inhibited the response of primary cortical cells to acetylcholine at low concentrations (10 pM) at 15 seconds

following exposure to the peptide and acetylcholine. This study shows that the τ -conotoxin peptide act at the acetylcholine receptor.

EXAMPLE 5

Effect of τ -Conotoxins in a Pain Model

[00056] The effect of τ -conotoxin peptides for use in treating pain was by testing in two pain models, the first being the hind-paw licking model (Woolfe and MacDonald, 1944; Plummer et al., 1991; Suh et al., 1992; Plone et al., 1996) and the second being the accelerating roto-rod model. In the hind-paw licking model, it was found that 10 nmol of τ -conotoxin peptide AuVA increased the latency to initiate hind-paw licking in mice on a 55° hot plate 15 minutes following freehand i.c.v. injection. It was further found that 1nmol τ -conotoxin peptide AuVA did not have any effect in this model. In the accelerating roto-rod model, it was found that τ -conotoxin peptide AuVA produced impairment of motor performance following injection of τ -conotoxin peptide AuVA. The effects seen in these models demonstrates that the τ -conotoxin peptides have analgesic properties.

[00057] It will be appreciated that the methods and compositions of the instant invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent to the artisan that other embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

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